CHARACTERISTICS OF THE 5S rRNA FROM COTTON SEEDS

A. M. Érmatov, I. T. Yakubov,
and R. N. Nuriddinov

UDC 547.963.3:633.51

The individuality of the 5S rRNA from cotton seeds has been determined by the production of a single narrow band on gel electrophoresis and by the characteristic UV spectrum with values of the ratios E_{260}/E_{230} and E_{260}/E_{280} of 2.03-2.1 and 2.08-2.14, respectively. The melting curve of the 5S rRNA has been obtained and a total hyperchromic effect of 25% has been calculated. The nucleotide composition of the 5S rRNA has been established by determining the amount of nucleotides in an alkaline hydrolysate (22.6% of AMP; 30.2% of GMP; 22.6% of UMP; and 24.6% of CMP). The nucleotides were identified from the position of their appearance in the elution profile, from their UV spectra in 0.1 N HCl and 0.1 KOH, and also by one- and two-dimensional chromatography of the nucleotides obtained, in the presence of markers.

Criteria of the purity of nucleic acid preparations are their characteristic UV spectra with λ_{max} 260 nm and λ_{min} 230 nm, the magnitudes of the ratios E_{260}/E_{230} and E_{260}/E_{280} in preparations of nucleic acids freed from polysaccharide and protein impurities, which are generally two and more [1], and also the presence of a single band on electrophoresis in polyacrylamide gel (PAG) under normal conditions.

On recording the spectral absorption curve in UV light of a preparation of the 5S rRNA of cotton seeds purified by preparative gel electrophoresis and concentrated on a column of DEAE-cellulose [2] we obtained characteristic UV spectra. The values of the ratios E_{260}/E_{230} and E_{260}/E_{280} were 2.03-2.1 and 2.08-2.14, respectively.

On electrophoresis in 10% PAG in Tris-acetate buffer, pH 7.2 [3], and Tris-borate buffer, pH 8.3 [4], the preparation of the 5S rRNA gave only one band. The preparation also gave only one band after being kept under denaturing conditions: 0.01 M Na_2EDTA, 60°C, 5 min; 7 M urea, 60°C, 5 min. When gels with the denatured and renatured (0.02 M $Mg(CH_3COO)_2$, 60°C, 5 min, slow cooling) 5S rRNA were compared, it was observed that the renatured form migrated in the gel in the form of a narrower band than the denatured form.

By using a method of analysis more sensitive than visual observation — scanning — in the electrophoresis of denatured 5S rRNA some authors have observed conformational isomers differing in their electrophoretic mobilities [5].

It is interesting to note that when 5S rRNA from bakers' yeast was denatured its electrophoretic mobility became equal to that of the 5S rRNA of the cotton plant, although under normal conditions these magnitudes are different. The denaturation of the 5S rRNA is accompanied by a breakdown of the ordered helical sections and is characterized by an increase in the optical density of the solution — a hyperchromic effect. We have obtained information on the

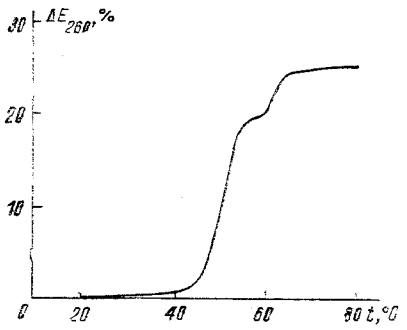

Fig. 1. Melting curve of the 5S rRNA from cotton-plant seeds in 0.15 M NaCl in 0.1 M phosphate buffer, pH 7.0. ΔE_{260}, % — relative increase in the optical density at λ_{260}.

Institute of the Chemistry of Plant Substances of the Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 817-821, November-December, 1980. Original article submitted June 2, 1980.

0009-3130/80/1606-0587$07.50

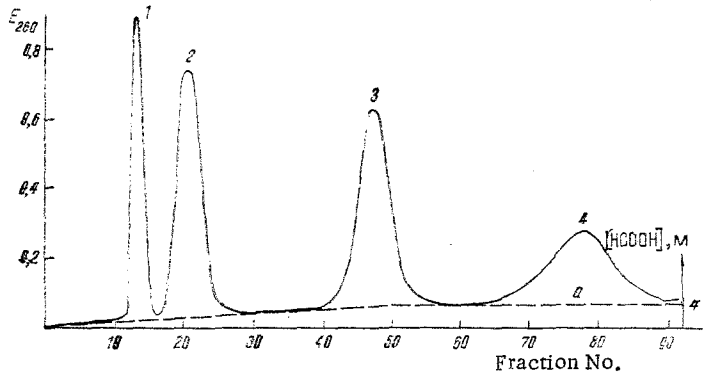

Fig. 2. Elution profile of the nucleotides in the chromatography of an alkaline hydrolysate of the 5S rRNA on a column of Dowex 1×8 (0.8 × 50 cm), 100-200 mesh, $HCOO^-$ form.

dependence of the optical density of a solution of 5S rRNA on the temperature, on the basis of which a melting curve has been plotted (Fig. 1).

A stepwise melting curve has been reported previously [7]. It indicates that the 5S rRNA molecule has several double helical sections the melting profiles of which do not overlap. The total hyperchromic effect amounts to 25%, which agrees with the figure given in the literature [8].

One of the important characteristics of a nucleic acid preparation is its nucleotide composition. To determine the nucleotide composition the nucleotides are separated by paper electrophoresis [9], thin-layer and paper chromatography [10, 11], and ion-exchange chromatography on columns in formic acid [12] or hydrochloric acid [13] systems.

Anion-exchange chromatography in columns permits the separation of the nucleotides more rapidly and with higher accuracy. Consequently, we separated the nucleotides from an alkaline hydrolysate of the 5S rRNA on a column of Dowex 1×8 (0.8 × 50 cm), 100-200 mesh, in the formate form. The nucleotides were eluted with a linear concentration gradient of formic acid (0-4 M), the total volume of the gradient being 200 ml. After the end of the gradient, elution was continued with a 4 M solution of HCOOH at a rate of passage of the eluent of 0.8 ml/min. The nucleotides were eluted from the column in the following sequence: peak 1 — 3'(2')-cytidylic acid; 2 — 3'(2')-adenylic acid; 3 — 3'(2')-guanylic acid; 4 — 3'(2')-uridylic acid (Fig. 2).

The fractions corresponding to a single peak were combined, the concentration of nucleotide in the solution was determined, and after the necessary calculation [14], the percentage concentration (mole %) was determined.

	AMP	GMP	UMP	CMP	Purines/pyrimidines	GMP + UMP / AMP + CMP	GMP + CMP / AMP + UMP
Cotton-plant 5S rRNA	22.6	30.2	22.6	24.6	1.11	1.11	1.21

In calculating the amounts of nucleotides, we took account of the background absorption of the formic acid itself. The rise in the optical absorption of the formic acid (background) with an increase in this concentration is clearly shown in Fig. 2 (curve a).

To identify the nucleotides we recorded the spectra [15] in 0.1 N HCl and in 0.1 N KOH (Fig. 3).

A nucleotide map of an alkaline hydrolysate of the 5S rRNA was obtained by two-dimensional chromatography in a thin layer of cellulose (FND, GDR), in the following systems: direction I) isopropanol—water (70:30, v/v) and NH_3 in the gas phase; direction II) isobutyric acid—0.5 N NH_4OH (10:6), pH 3.7 [10].

The spots were identified by comparison with markers. In the first direction the alkaline hydrolysate was separated into two spots: an upper spot containing a mixture of AMP, CMP, and UMP, and a lower spot consisting of GMP. In the second direction the upper spot was fractionated into its components: 1) 3'(2')-AMP; 2) 3'(2')-CMP; 3) 3'(2')-UMP. The R_f values of spots 3 and 4 (3'(2')-GMP) were almost identical.

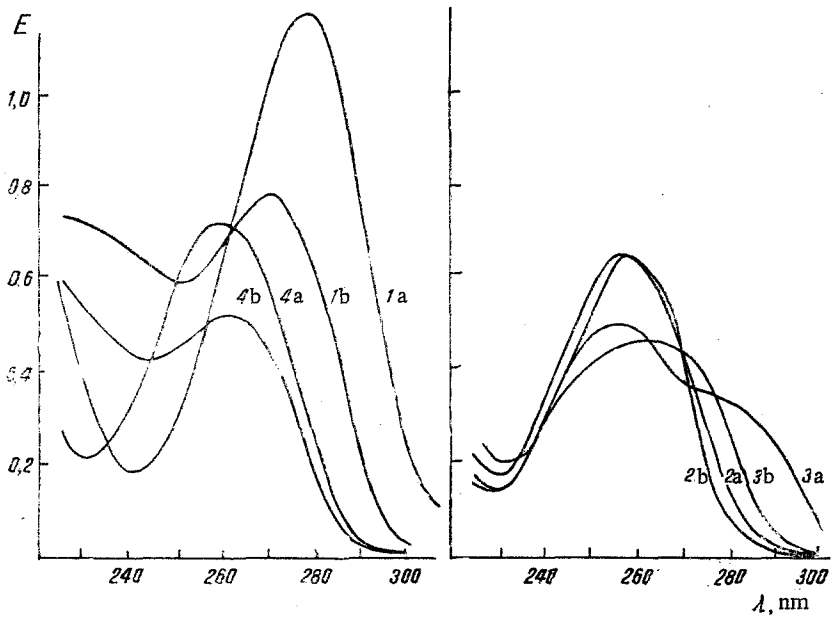

Fig. 3. UV spectra of the fractions obtained (1-4) in 0.1 N HCl (1a-4a) and in 0.1 N KOH (1b-4b).

EXPERIMENTAL

The cottonseed 5S rRNA was isolated and purified by the method described previously [2]. The spectrometric measurements were performed on an SF-4 instrument.

Thermal denaturation at temperatures of 20-80°C was studied on an SF-4 instrument fitted with a thermostating block connected with an ultrathermostat. The cell was filled with a solution of the 5S rRNA with a concentration of 1 OU/ml in 0.1 M NaCl in 0.01 M phosphate buffer, pH 7.0, and the control cell was filled with a solution of the 5S rRNA with a concentration of 1 OU/ml in 0.1 M NaCl in 0.01 M phosphate buffer, pH 7.0, and the control cell was filled with the buffer alone. The optical density at λ_{260} was measured at intervals of 5°C 10 min after the given temperature had been reached. From the results obtained a melting curve was plotted and the hyperchormic factor was determined.

For electrophoresis we used the following buffer solutions: 2 liters of 0.04 M Tris, 0.02 M CH_3COONa, 0.002 M disodium salt of EDTA, pH 7.2; 2 liters of 0.09 M Tris, 0.002 M Na_2EDTA, 0.001 M $Mg(CH_3COO)_2$, pH 8.3 (adjusted with boric acid). 10% PAG was prepared as described previously [2]. Electrophoresis was performed at 5 mA per tube in each of which one OU of 5S rRNA in a volume of 50 µl was deposited.

Denaturation was carried out in the same buffer solutions with the addition of the disodium salt of EDTA to a concentration of 0.01 M or urea to 7 M. A solution of 5S rRNA in a particular buffer solution was kept for 5 min in a water bath heated to 60°C and was then rapidly cooled with ice to 0°C.

For renaturation we used part of the solution (1-2 OU in 50 µl) containing denatured 5S rRNA (0.01 M Na_2EDTA, 60°C, 5 min) to which an equal volume of 0.02 M $Mg(CH_3COO)_2$ had been added, and kept it at 60°C for 5 min and then slowly cooled it to room temperature.

Electrophoresis was performed under the same conditions, but in the case of urea the polyacrylamide gels in the tubes were prepared in the presence of urea in a concentration of 4 M. After electrophoresis the gels were left to stain in dye (acridine orange with lanthanum acetate in acetic acid) for 16 h, and the excess of dye was washed out with hot water.

To determine its nucleotide composition, the 5S rRNA (1-2 mg) was dissolved in 1 ml of 0.3 N KOH and the solution was kept at 37°C for 18 h. The cooled hydrolysate was treated in drops with cooled concentrated $HClO_4$ to pH 8. The resulting solution was kept in ice for 1 h and the precipitate of $KClO_4$ that formed was separated off with centrifuging. The hydrolysate obtained was deposited on a column (0.8 × 50 cm) of Dowex 1×8 100-200 mesh, which had first been converted into the formate form and washed with boiled-out double-distilled water to the neutral pH. After deposition, the column was washed with water to $E_{260} = 0$ at which the nucleoside fraction formed from the 3'-end of the 5S rRNA molecule is eluted [16].

The nucleotides were eluted with a linear concentration gradient of formic acid (0-4 M). The mixer was charged with boiled-out double-distilled water (100 ml) and the reservoir with 100 ml of a 4 M solution of HCOOH. After the end of the gradient solution, elution was continued with a 4 M solution of HCOOH at the rate of 0.8 ml/min; 4-ml fractions were collected and their optical densities at λ_{260} were measured, and then a graph of the elution profile was constructed (see Fig. 2). The fractions corresponding to a single peak were combined, the concentrations and volumes were determined, the nucleotide composition was calculated [14], and the results obtained were entered in a table. Each combined peak was evaporated in a rotary evaporator at 35-38°C until the formic acid had been eliminated completely (2-3 times, to a neutral reaction). The fractions obtained were used for the identification of the separated nucleotides.

The nucleotides were identified on the basis of the following facts:

1) emergence in the elution profile (comparison of the results obtained with literature information and with profiles of chromatography performed under our conditions with an alkaline hydrolysate of a commercial preparation of yeast RNA);

2) the UV spectra of the nucleotides in 0.1 N HCl and 0.1 N KOH, the absorption ratios E_{250}/E_{260}, E_{270}/E_{260}, E_{280}/E_{260}, E_{290}/E_{260} and a comparison of the results with literature information [15] taking permissible deviations into account; and

3) characterization of the separate fractions with the aid of one- and two-dimensional thin-layer chromatography on FND cellulose in the systems isopropanol—HCl—water (170:41:39), isopropanol—NH_3—water (70:10:20), and ethanol—1 M CH_3COONa, pH 7.5 (75:30) direction I — isopropanol—water (70:30) and NH_3 in the gaseous medium; direction II — isobutyric acid—0.5 N NH_4OH (10:6), pH 3.7.

The plates with the FND cellulose were prepared in accordance with the instructions attached to it.

Curve *a* was obtained in a blank experiment, i.e., as the result of the passage of the concentration gradient of formic acid through the column without nucleotides (Fig. 2).

SUMMARY

It has been established that the 5S rRNA preparation is pure and electrophoretically homogeneous.

The hyperchromic effect has been calculated and the characteristic melting curve of the 5S rRNA has been obtained.

The nucleotide composition of the 5S rRNA has been determined.

LITERATURE CITED

1. Techniques of Biochemical Investigation of Subcellular Structures and Biopolymers [in Russian], Minsk (1977).
2. A. M. Ermatov and R. N. Nuriddinov, Khim. Prir. Soedin., 489 (1978).
3. H. Maurer, Disc-Elektrophorese, Theorie und Praxis, Walter de Gruyter, Berlin (1968).
4. G. R. Philipps and J. L. Timko, Anal. Biochem., 45, No. 1, 319 (1972).
5. M. B. Kel've, A. Kh. Metopalu, A. Ya. Lind, M. Yu. Saarma, R. L. Villems, Mol. Biol., 12, No. 3, 695 (1978).
6. A. M. Ermatov, I. T. Yakubov, and R. N. Nuriddinov, Khim. Prir. Soedin., 56 (1979).
7. R. L. Villems and A. Ya. Lind, Uch. Zap. Tartusk. Inst., No. 285, 93 (1971).
8. G. Soave, E. Galante, and G. Torti, Bull. Soc. Chem. Biol., 52, No. 8, 857 (1970).
9. B. F. Vanyushin and A. N. Belozerskii, Dokl. Akad. Nauk SSSR, 127, 455 (1959).
10. A. I. Krutilina, A. D. Mirzabekov, T. V. Venkstern, and A. A. Baev, Biokhimiya, 30, No. 6, 1225 (1965).
11. V. K. Vasil'ev, Nauch. Dokl. Vyssh. Shk., Biol. Nauk, 9, 118 (1971).
12. G. M. Shavlovskii, E. M. Logvinenko, and M. H. Stasiv, Biokhimiya, 34, No. 1, 150 (1969).
13. G. G. Revich, R. T. Toguzov, and K. I. Mordukhovich, Biokhimiya, 38, No. 1, 87 (1973)
14. V. G. Konarev and S. D. Tyuterev, Methods of the Biochemistry and Cytochemistry of Plant Nucleic Acids [in Russian], Leningrad (1970).
15. T. V. Venkstern and A. A. Baev, Absorption Spectra of Minor Components and Some Oligonucleotides of RNAs [in Russian], Moscow (1967).
16. V. A. Erdmann, Mucl. Acids Res., 6, No. 1, r. 29 (1979).